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NOTICE OF MEETING AND CALL FOR ABSTRACTS

26TH PLANT DEVELOPMENT WORKSHOP

SATURDAY, NOVEMBER 2, 1991

MCMASTER UNIVERSITY, HAMILTON

Hosted by Dr. J.N.A. Lott, Dr. E. Weretilnyk, Dr. Irene Ockenden and Ms. Thelma Leech (Biology Department, McMaster University)

You and your colleagues are invited to attend this special Workshop which will recognize Dr. Douglas Davidson's efforts in initiating the Plant Development Workshops in 1977.

Preliminary Program:

8:45 - 9:20 am	Registration in Life Sciences Bldg. Rm. 213
9:20 - 12:00	Special lecture by Dr. D. Davidson and invited speakers Dr. S. Gleddy and Dr. B. McKersie
12:00 - 1:00 pm	Lunch and poster session
1:00 - 2:00 pm	Invited speakers Dr. R.L. Peterson and Dr. E. Weretilnyk
2:00 - 4:00 pm	Contributed papers
4:00 - 4:45 pm	Reception

Presentations:

Please submit an abstract typed in a box 17 cm wide by 10 cm deep. Give title, author, address and text. Please indicate clearly if you wish this to be a talk or a poster presentation. Talks will be limited to 15 minutes. **Deadline for submission of abstracts ..... Tuesday, October 15, 1991.**

Send Abstracts To:

Dr. Irene Ockenden
Department of Biology
McMaster University
1280 Main Street West
Hamilton, Ontario, L8S 4K1

Telephone: (416) 525-9140
ext. 2087 or 3538

FAX No.: (416) 521-2955

Information Requested:

Please let us know the number of people from your lab who will be attending the workshop so that we can make appropriate lunch arrangements. Please let us know if you are willing to host the next workshop.

(26)

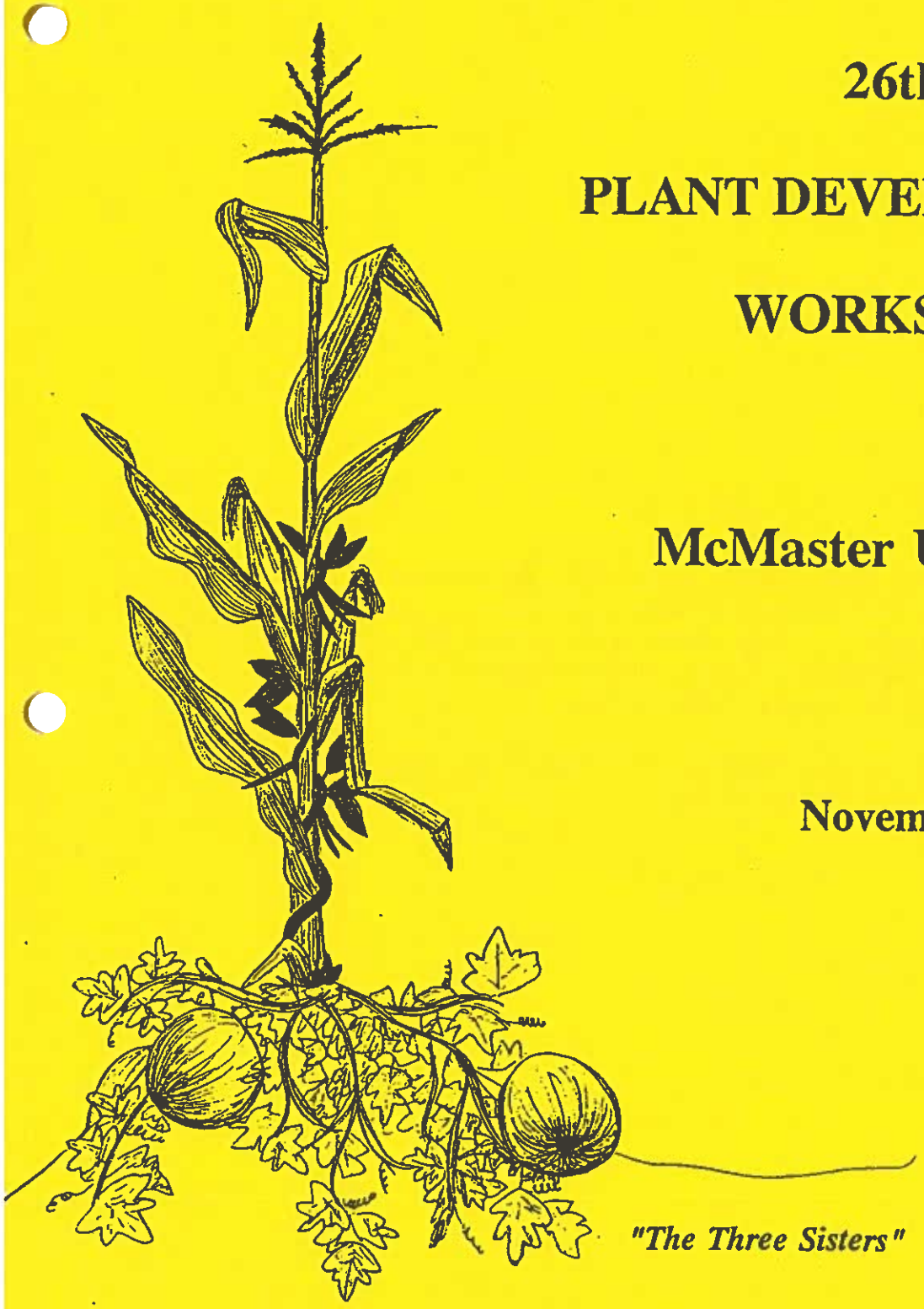
26th

PLANT DEVELOPMENT

WORKSHOP

McMaster University

November 2, 1991



"The Three Sisters"

"The Three Sisters" were a trinity of deities who served as the guardian spirits of corn, beans and squash. In Iroquois agriculture, a corn seed, bean seed and squash seed were traditionally planted together and the products of the harvest were eaten together. This represents an early form of local 'agricultural biotechnology', the wisdom of which we are beginning to appreciate in the practice of intercropping.

[Reference: R.W. Waugh, Iroquois Foods and Food Preparation. Memoir 86, Canada Dept. of Mines, Geological Survey, Reprint 1973.]

26th PLANT DEVELOPMENT WORKSHOP NOVEMBER 2, 1991 McMASTER UNIVERSITY

8:45 - 9:20	Registration, coffee, poster set-up Life Sciences Building, Room 213
9:20 - 9:30	Opening welcome, McMaster Medical Centre, Room 1A05
9:30 - 10:10	**D.D. Davidson.** The role of cell division in plant development.
10:10 - 10:50	**S. Gleddie.** Broadening the gene pool through protoplast fusion.
10:50 - 11:10	Coffee
11:10 - 11:50	**B.D. McKersie.** Somatic embryogenesis and artificial seeds.
11:50 - 1:00	Lunch, poster session, Life Sciences Building
1:00 - 1:30	**E. Weretilnyk.** Plant development under stress.
1:30 - 2:00	**R.L. Peterson.** Mycorrhizal fungi and root development.
2:00 - 2:15	**C.M. Kampny and T.A. Dickinson.** Quantitative description of floral development in Veronica chamaedrys and Veronicastrum virginicum (Scrophulariaceae).
2:15 - 2:30	**H. Stewart and J.M. Canne-Hilliker.** Alteration of breeding system in Agalinis (Scrophulariaceae).
2:30 - 2:45	**J.M. Canne-Hilliker and R.W. Scribailo.** Floral ontogeny in Torenia fournieri (Scrophulariaceae).
2:45 - 3:00	**J. Zhao and D.H. Simmonds.** Induction of microspore embryogenesis of Brassica napus cv. Topas: effect of induction duration, plant age and seasonal variation.
3:00 - 3:15	**A.M. Zobel and S.A. Brown.** Localization of furanocoumarins in intercellular spaces.
3:15 - 3:30	**A.M. Zobel, S. Louis, A. Keightly, M. Podbielkowska and M. Waleza.** Furanocoumarins as antimitotics.
3:30 - 3:45	**X. Zheng and R.B. van Huystee.** Oxidation of feruloyl pentosan and free ferulic acid as a function of peanut (Arachis hypogaea L.) peroxidase isozymes.
3:45 - 4:00	**F.C. Guinel.** Improved nodulation in two low nodulating mutants by treatment with ethylene antagonists.
4:00 - 4:15	**L.W. Stobbs, A.B. Broadbent, W.R. Allen and A.L. Stirling.** Transmission of tomato spotted wilt virus by the western flower thrips to weeds and native plants found in Southern Ontario.
4:15 - 5:00	Reception, Life Sciences Building, Room 213

ABSTRACTS OF PAPERS

D.DAVIDSON. Cell Division in Plants. Biology Dept., McMaster University. Cell division in higher plants is generally a highly ordered event:it is controlled in position in the plant body, in frequency and in the orientation of both the axes of division and of cell expansion that occurs in a cell division cycle. Two features of plant structure determine the typical patterns of cell division:1) the development, initiated early in embryogenesis, of differentiated and generally dead cells that comprise the central core of all organs;2) the presence of cell walls that lock cells into fixed positions and prevent movement.These features determine that almost all divisions occur at the periphery of the plant and that new cells are along axes away from the central core of differentiated cells.In all cases, the proliferative phase of a mitotically active clone of cells is limited, suggesting that plants impose strict controls on the frequencies of division and on entry into a non-cycling phase. These controls continue to work in cultures,presenting problems for tissue culture systems. The possible nature of these controls will be discussed.

Broadening the gene pool through protoplast fusion. S. Gleddie, Agriculture Canada, Plant Research Centre, Ottawa, Ontario K1A 0C6.

Since the first somatic hybrid plants were produced in 1972, several advances have increased the efficiency and usefulness of this technology. New developments include novel, more efficient selection procedures, methods to direct the partial elimination of one genome, and improvements in plant regeneration protocols for many important crop species. These will all be discussed with reference to work at the Plant Research Centre on the transfer of agronomically important genes from sexually-incompatible wild relatives into such crop species as tobacco, tomato and canola. The potential applications of this research to plant science and some possibilities for the future will be discussed.

SOMATIC EMBRYOGENESIS AND ARTIFICIAL SEEDS

Bryan D McKersie, Dept of Crop Science, University of Guelph

Dry somatic embryos or artificial seeds are are being developed as an alternative method of vegetative plant propagation with the advantage of long term storage. Somatic embryos are analogous in many ways to zygotic embryos and pass through globular, heart and torpedo shaped developmental stages. They also can be induced to continue in their development in a manner similar to a seed and deposit storage reserves and eventually acquire desiccation tolerance. In Medicago sativa, somatic embryos are induced from the subepidermal cells of petiole explants on media containing the auxin, 2,4-D. Synchrony of embryo development is achieved by first dispersing the petiole-derived callus in liquid suspension and then sieving the suspension to a uniform size. Embryo development is initiated on hormone-free media. Once the embryo reaches the late torpedo stage of development it will germinate precociously, unless it is exposed to abscisic acid (ABA) or high sucrose. The embryo accumulates storage proteins and carbohydrates when late torpedo stage embryos are transferred to high sucrose media containing glutamine and low levels of ABA. Desiccation tolerance is induced by subsequent treatment with high levels of ABA and then the embryos are subsequently dried to 10-15% moisture. Embryos have been stored for as long as 2 years in the dry state. Germination of dry somatic embryos requires careful rehydration to over 20% moisture followed by rehydration on moist filter paper and transfer to soil.

Artificial seeds have many commercial applications and the possibility exists to establish a Canadian based industry. In order for us to transfer this process easily to other plant species we require inprovements in our basic understanding of seed and somatic embryo development, of the physiological processes involved in control of somatic embryo initiation, of storage protein and carbohydrate synthesis, of the expression of desiccation tolerance, and of the germination process in dry somatic embryos.

Plant development under stress.

E. Weretilnyk, Department of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

Many plants have evolved developmentally regulated mechanisms to adapt to conditions of environmental stress. These developmental changes reflect morphological, physiological, and metabolic adjustments all of which are ultimately orchestrated by the expression of information which resides at the genetic level. While much effort has been directed towards breeding for specific developmental traits which confer stress tolerance in plants, these activities have been largely done on the level of the whole organism. More recently, the promise of genetic engineering in plants has led towards a growing interest in the molecular identification of "stress tolerance" genes. This shift arises, in part, in response to the relative ease of plant transformation and the potential this technology brings to specifically altering developmental events, structural features, physiological and metabolic pathways once the molecular basis underlying these activities has been identified and characterized. Our objective, therefore, is to identify aspects of plant development under stress which are both amenable to genetic engineering and which will ultimately result in increased tolerance of plants towards environmental stress. Molecular approaches towards the study of stress tolerance in higher plants, specifically with respect to cold acclimation and osmotic stress, will be discussed.

MYCORRHIZAL FUNGI AND ROOT DEVELOPMENT.

R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1.

Roots of the majority of vascular plants are colonized by symbiotic fungi to form mycorrhizae. Mycorrhizae have been categorized into types based partly on the fungal symbionts involved and partly on the structural features that result from the interaction between the symbionts. Although vesicular-arbuscular mycorrhiza is the most prevalent type, the changes in root morphology and structure are not obvious. Alterations in the mitotic cycle in the apical meristem, decondensation of root cell nuclear chromatin, and an increase in plasma membrane surface area and cytoplasm in cells containing arbuscules have been reported. In ectomycorrhizae, the fungal symbiont can induce profound alterations in root morphology including repeated dichotomous branching in Pinus spp., precocious lateral root initiation, and proliferations of lateral roots which become encased in a peridium-like fungal sheath to form 'tubercles' in Pseudotsuga, Eucalyptus and a few other genera. In some angiosperm species, ectomycorrhizal fungi induce marked radial enlargement of epidermal cells whereas in other species, wall ingrowths may be induced in epidermal cells. The morphology of the fungal hyphae in the Hartig net and mantle also undergo striking structural changes. The more specialized mycorrhizal types, ectendomycorrhiza and arbutoid mycorrhiza have characteristics in common with ectomycorrhiza but fungal penetration into root cells occurs.

Quantitative description of floral development in Veronica chamaedrys and Veronicastrum virginicum (Scrophulariaceae). C. M. KAMPNY* & T. A. DICKINSON. Botany Departments, Royal Ontario Museum and University of Toronto, Toronto ON.

Flowers of Veronicastrum are intermediate between generalized Scrophulariaceous flowers (e.g. Agalinis, Digitalis) and those of Veronica. Development in Veronica and Veronicastrum was studied to elucidate the growth patterns responsible for differences between their mature flowers. Floral parts were measured throughout development; significant stages were recorded with the SEM. Plots of measurements versus bud length (as a proxy for biological age) show that the calyx lobes of Veronicastrum enlarge earlier; but are later surpassed by those of Veronica. This is also the case with the corolla lobes. The corolla tube is larger in Veronicastrum from its inception. The early growth of the anthers in Veronica is a specialized feature. In both taxa stamen filaments and the style elongate late in development. Measurements were also summarized using principal components analysis (PCA). Developmental trajectories in the space of PC1, PC2, and PC3 differ from the earliest stages on, mirroring differences observed with the SEM. PCA can thus be used to portray measurable differences in floral development patterns, facilitating comparisons of several taxa at once.

Alteration of breeding system in Agalinis (Scrophulariaceae).

Heather Stewart and J.M. Canne-Hilliker. Department of Botany, University of Guelph, Ontario.

Agalinis are primarily out-crossing, insect pollinated annuals in North America. Most species are visited by bees which are accommodated by the pink or rose coloured, campanulate corollas. The abaxial corolla throat is yellow lineate and red spotted. Prior to anthesis the style is bent sharply so that the decurrent stigma is tucked down into the abaxial side of the corolla. At anthesis the style straightens and the stigma is exserted beyond the orifice of the corolla. Agalinis paupercula is found from southern Ontario to Nova Scotia and has flowers of the type described above. Agalinis neoscotica also occurs in Nova Scotia, and is generally differentiated from A. paupercula by its diminutive habit, proportionally longer calyx lobes, and wider leaves. Several features of the mature flowers indicate that A. neoscotica may be primarily autogamous. A preliminary investigation of developmental morphology showed that the orientation of the anthers to stigma differs from that seen in other Agalinis. Dissection of the buds prior to anthesis shows that the anthers are held adjacent to the stigma and that they dehisce before the corolla opens. Pollen/ ovule ratios are lower in A. neoscotica. A transplant garden containing plants of A. neoscotica and A. paupercula has been established at Guelph so that controlled pollinations can be made.

Floral ontogeny in Torenia fournieri (Scrophulariaceae). J.M. Canne-Hilliker and R.W. Scribailo, University of Guelph, Ontario and Purdue University North Central, Indiana.

We have chosen Torenia fournieri as a model organism to investigate genetic aspects of floral morphogenesis. We report findings from SEM and anatomical studies of wildtype floral ontogeny. Sepal primordia arise nearly simultaneously and fold over the apex as they elongate. Three abaxial petal primordia arise on the flanks of the apex as the two unequal pairs of stamen primordia appear. Two unequal carpel primordia form slightly before or concurrently with those of the adaxial petal complex. Petal primordia remain small as stamens and gynoecium enlarge. Interprimordial growth forms a shallow corolla tube very early. As thecae form in the stamens, a nectary becomes evident, and the adaxial petal complex becomes keeled. Stigma lobes differentiate prior to style formation at about the time of ovule enlargement. Stamen development is complex. Within an anther, thecae are unequal in length. In each theca the locule adjacent to the filament is smaller. The walls of the small locules elaborate to form a longitudinal flange that projects toward the centre of the bud. Thecae of an anther differentiate in the usual parallel orientation, but the connective becomes markedly broader so that the thecae come to lie apex to apex. In this linear arrangement the thecal flanges are positioned on the periphery (to the outside) of each anther pair. Filaments of the abaxial stamen pair curve up and inwardly as they elongate. This brings their anthers together so the tandemly arranged thecae of each anther lie side by side above the stigma. Surface cells of adjacent anthers within a pair interdigitate. The abaxial pair of anthers (above the stigma) mature first, followed by the adaxial pair below the stigma. At anthesis (but not before the flower opens) pollen is ejected when pressure is applied to the flanges of the thecae.

INDUCTION OF MICROSPORE EMBRYOGENESIS OF BRASSICA NAPUS CV. TOPAS: EFFECT OF INDUCTION DURATION, PLANT AGE AND SEASONAL VARIATION

Jiping Zhao and Daina H. Simmonds, Plant Research Centre, Agriculture Canada, Ottawa, Ontario, K1A 0C6

Isolated microspores of B. napus cv. Topas can be induced to switch from pollen development to embryo development by exposure to a heat treatment of 32.5°C. The frequency of embryogenesis is related to the stage of microspore development. The highest frequency of embryogenesis is obtained from microspore isolations where the majority is late uninucleate (LU, after the vacuole has started to break down) or early binucleate (B). Lower frequencies of embryogenesis are obtained from isolations where the majority of microspores are late uninucleate vacuolate (LUV, prior to vacuolar breakdown). The duration of heat treatment required for embryogenesis induction is stage dependent. The frequency of embryogenesis fluctuates seasonally and varies with the donor plant age. These data will be discussed in light of the induction process.

Localization of furanocoumarins in intercellular spaces

Alicja M. Zobel and Stewart A. Brown
Department of Chemistry, Trent University, Peterborough, ON K9J 7B8

Furanocoumarins, known as antimicrobial agents, have been located on the surface of leaves, fruits and seeds of many plants belonging to the Rutaceae and Umbelliferae. On *Ruta graveolens* leaves the surface concentration of furanocoumarins reaches very high absolute amounts, up to 2 mg/g fresh weight, measured by HPLC after being removed from the surface by dipping in boiling water for 1 sec. This method was proved not to damage the epidermal cells. The percentage on the surface was never higher than 86%, and prolonged time of dipping did not increase the percentage concentration on the surface, indicating that 14% of the furanocoumarins are located within the leaf. After the epidermis had been peeled off and the intercellular spaces washed with acetonitrile, HPLC revealed furanocoumarins in the extracts. Callus cells of *R. graveolens* contained furanocoumarins on the cell surface, indicating that not only epidermal cells extrude these compounds. Imprints in dental wax of living cells of the mesophyll after the epidermis had been peeled away revealed in the scanning electron microscope crystal-like deposits on the surface of the cells bordering the intercellular spaces. Thus extrusion of toxic compounds, potentially harmful to the cytoplasm of the cells producing them, can be sent outside the cells to their surfaces, either the intercellular spaces or the surface of the cuticle.

FURANOCOUMARINS AS ANTIMITOTICS

Alicja M. Zobel,[1] Sharon Louis,[2] Angela Keightly,[2] Maria Podbielkowska[3] and Maria Waleza[3]
Departments of Chemistry[1] and Biology,[2] Trent University, Peterborough, ON K9J 7B8 and Plant Anatomy and Cytology, Warsaw University,[3] 00-927 Warsaw, Poland

In darkness and under UV, two plant furanocoumarins affected mitoses and morphology of mitotically active cells. Embryonic meristem of *Allium cepa* root tips and animal cell cultures showed fewer mitoses, but more chromosomal aberrations and blockage of some phases of mitosis. Even after 1h in the presence of UV, psoralen and xanthotoxin caused abnormal chromosome separation leading to chromosomal aberrations in sister cells, and complete cessation of mitosis after 3h. In darkness mitotic spindle aberrations were visible after 3h, but the primary mechanism after 1h was on the endomembrane system. The first responses were in the cell membrane, nuclear envelope, and endoplasmic reticulum, changing their structure and causing fragmentation. We suggest that although the effect of UV is on DNA, as is well-known, the primary reaction, which occurs in darkness, is on the endomembrane system.

Oxidation of Feruloyl Pentosan and Free Ferulic Acid as a Function of Peanut(*Arachis hypogaea* L.) Peroxidase Isozymes

X. Zheng and van Huystee R.B.

Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7

ABSTRACT

In the range of 2 to 8 mg/ml, the viscosity of wheat flour pentosan solution was increased by the addition of anionic or cationic peroxidase together with H_2O_2. When the pentosan solution reached 10 mg/ml, a gel was formed(gelation) upon oxidation. The gelation was dependent on both enzyme concentration and pH. The subsequent saponification of the gel resulted in the production of free ferulic and diferulic acid which have been identified by silica gel thin layer chromatography. Furthermore, commercial ferulic acid was oxidized to diferulic acid and higher polymerzed products by peroxidase isozymes. The pH and reaction-period had an effect on the oxidation pattern. On the other hand, pectin from apple and commercial deesterified xylan and polygalacturonic acid did not form a gel even in the presence of peroxidases. Isolated peanut cell wall can also cause gelation and free ferulic acid-oxidation probably due to peroxidase in the cell wall. Antibodies against peroxidase isozymes acted as specific inhibitors because the preincubation of these antibodies with peroxidases and cell wall leads to the loss of oxidative capacity. Consequently, peroxidase isozymes play a considerable role in cell wall rigidification by regulating oxidative crosslink of wall structural components such as feruloyl pentosan.

IMPROVED NODULATION IN TWO LOW NODULATING MUTANTS BY TREATMENT WITH ETHYLENE ANTAGONISTS. F. C. GUINEL, Department of Biological Sciences, Brock University, St. Catharines, Ontario, L2S 3A1

The infection process in the lateral roots of Pisum sativum (L.) cv Sparkle was compared to that of two low nodulating mutants, E2 (sym 5) and E107 (brz). Longitudinal hand-sections of comparable segments of lateral roots from 18 day-old plants were observed by light microscopy. In Sparkle, about 25% of the infections terminated in the epidermis, a similar number stopped in the cortex, while 50% resulted in the formation of a nodule primordium or an emerged nodule. The mutant E2 (sym 5) was infected as often as was the parent: they both had on average 6.5 infections per cm of lateral root. The number of infections in E107 (brz) was only a third that of "Sparkle". The two mutants were able to form normal infection threads, i.e. infection threads indistinguishable from those of the parent. In E107, most of the infections aborted in the epidermis, thus in only 14% of the infections did the infection thread penetrate beyond the epidermis. In E2, cell divisions rarely occurred in advance of the infection thread (5%) and few nodule primordia were produced. Nodulation of both mutants was partially restored by treating the plant with the ethylene inhibitor silver. Silver also increased the number of emerged nodules in "Sparkle". It seems to act principally at the epidermis: when the 2 mutants and the parent were treated, less infection threads were seen arrested in this cell layer, and more were found in later stages. In addition, in silver-treated E2, infection threads associated with cell divisions in the inner cortex were more numerous than in the controls. The involvement of ethylene in stimulating nodule development is proposed.

Transmission of Tomato Spotted Wilt Virus by the Western Flower Thrips to Weeds and Native Plants found in Southern Ontario.
L.W. Stobbs, A.B. Broadbent, W.R. Allen, and A.L. Stirling, Agriculture Canada, Research Station, Vineland Station, Box 6000, Ontario, Canada, L0R 2E0.

Weed species and native plants occurring in southern Ontario, grown from seed or collected from the field, were exposed in a growth room to natural transmission of tomato spotted wilt virus (TSWV) by the western flower thrips (WFT), Frankliniella occidentalis. These plants were assayed for TSWV by both enzyme-linked immunosorbent assay (ELISA) and bioassay on suitable indicators, and thrips reproduction was examined on TSWV-susceptible plants. Of 302 native plant species tested, 113 species representing 35 families were field susceptible to TSWV, 62 species of which are first reported hosts for this virus. Eighty-six percent of these plants were ovipository hosts for the WFT.

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CHANGES INDUCED BY WHEAT STREAK MOSAIC VIRUS

Sandy Kofalvi, Annette Nassuth, and Jian-guo Gao.
University of Guelph, Department of Botany, Guelph, Ontario N1G 2W1

Microscopic observations of wheat, barley, and triticale leaf tissues revealed several wheat streak mosaic virus-induced cytological changes which were further studied at the protein and nucleic acid levels, to investigate the role of these changes in host defence and/or virus replication. The deposition of a layer seemingly containing polyphenolics and some lignin and the appearance of holes in the cell walls, which at late infection stages collapse are features which compelled us to pursue this study. We are investigating the induction of enzymes similar to those involved in the defence mechanism shown for dicot plants. Specifically, enzymes involved in phenylpropanoid metabolism such as phenylalanine ammonia lyase, cinnamyl alcohol dehydrogenase, 4-coumarate lyase, and peroxidase have been tested for activity levels in infected versus mock inoculated plants.

INTERACTIONS BETWEEN ALFALFA NODULATION GENOTYPES AND VAM FUNGI.

S.M. Bradbury[1], R.L. Peterson[1] and S.R. Bowley[2]. [1]Department of Botany, [2]Department of Crop Science, University of Guelph, Guelph, Ontario, N1G 2W1.

Seedlings of a non-nodulating alfalfa (Medicago sativa L.) genotype (nod^-fix^-) and an ineffectively nodulating alfalfa genotype (nod^+fix^-) exhibited resistance to vesicular-arbuscular mycorrhizal (VAM) colonization, when grown under growth room conditions in pot cultures of turface inoculated with four Glomus species (Glomus fasiculatum (Thaxter) Gerdemann & Trappe emend. Walker & Koske, Glomus intraradices Schenck & Smith, Glomus monosporum Gerdemann & Trappe, Glomus versiforme (Karsten) Berch) and Gigaspora margarita Becker & Hall. Appressoria, showing considerable variation in size and shape, developed on the root surface, but internal structures such as arbuscules and vesicles failed to form. Wild-type (nod^+fix^+) alfalfa seedlings developed normal VAM colonization. Colonization of the three alfalfa genotypes occurred when seedlings were grown under greenhouse conditions in pots containing non-sterile soil from two local fields of alfalfa. This phenomenon will be extremely useful in studying the processes of recognition and compatibility between plant species and VAM fungal species.

POSTERS POSTERS POSTERS

ISOLATION OF THE ADENINE PHOSPHORIBOSYLTRANSFERASE GENE FROM *ARABIDOPSIS THALIANA*.

E. McWhinnie and B. A. Moffatt. University of Waterloo, Waterloo, Ontario N2L 3G1.

Adenine phosphoribosyltransferase is an enzyme of the purine salvage pathway which converts adenine and PRPP to AMP. Due to its central role in nucleotide metabolism, the gene for APRT, *apt*, is expressed in all tissues. Although *apt* is a well characterized housekeeping gene in animals, very few constitutively-expressed genes have been isolated from plants. Moreover, the promoters of such genes have not been well defined. As part of our study of the role of APRT in plant development, we are investigating the regulation and expression of *apt*. Here, we report the isolation and preliminary characterization of the first gene for APRT from a plant, *Arabidopsis thaliana*. Initially, a partial *apt* cDNA from *Brassica juncea* was isolated using oligonucleotides based on the amino acid sequence of the purified protein. The *B. juncea* cDNA was used to isolate the *apt* cDNA from *A. thaliana*. Subsequently, the gene for APRT was identified by screening an *A. thaliana* genomic library. Using various fragments of this cDNA as probes, a physical map of the *apt* gene was constructed. Sequencing analysis was undertaken to characterize promoter elements associated with this gene.

EXPRESSION OF ADENINE PHOSPHORIBOSYLTRANSFERASE DURING POLLEN DEVELOPMENT IN *ARABIDOPSIS*

Sharon M. Regan and Barbara A. Moffatt. Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

In order to understand the role of the purine salvage pathway in plants, we are examining a mutant deficient in adenine phosphoribosyltransferase (APRT), an enzyme responsible for the conversion of adenine to AMP. In *Arabidopsis*, the mutant is male sterile due to aborted pollen development. Cytochemical analysis has shown that the course of pollen development in the mutant begins to diverge from that in the wild type soon after the dissolution of the callose wall and the release of the microspores. The pollen wall does not develop normally and the appearance of the vacuole is irregular. Many of the mutant microspores fail to undergo mitotic divisions and the activities of two enzymes, alcohol dehydrogenase and esterase, are lost. Although the majority of the morphological changes in the mutant appear in the developing pollen, the region directly affected by the APRT deficiency could be any of the tissues of the anther. We are currently using *in situ* hybridizations to localize APRT transcripts during pollen development in an attempt to gain insight into the mechanism by which the APRT deficiency causes male sterility.

EMBRYOGENESIS FROM ISOLATED *BRASSICA NAPUS* MICROSPORES

TELMER, Cheryl A., NEWCOMB, William and SIMMONDS*, Daina H.
Department of Biology, Queen's University, Kingston, Ontario K7L 3N6.
*Plant Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6.

When *Brassica napus* cv. Topas microspores are isolated, heat treated and cultured, haploid embryos are produced instead of pollen. A narrow period in gametophytic development is susceptible to manipulation. Isolated microspores were developmentally staged and isolations selected for obtaining high frequencies of microspore-derived embryos. Samples for studies of cellular morphogenesis were fixed and embedded for light and transmission electron microscopy. *In vivo* microspore development progressed differently than *in vitro* cultured and heat treated microspores. Aspects of the cell walls, cytoplasmic division symmetry, nuclear condensation and starch content were affected. Several divisions occurred before a series of periclinal divisions distinguished the protoderm layer of globular embryos at around day 8 in culture. Globular embryos continued developing to form heart, torpedo and cotyledonary morphologies.

EMBRYONIC MUTANTS OF ARABIDOPSIS THALIANA ISOLATED FOLLOWING AGROBACTERIUM-MEDIATED SEED TRANSFORMATION. Deena Errampalli, Linda Castle, Brenda Stewart, Amy Tomlinson, and David Meinke. Oklahoma State University, Department of Botany, Stillwater, OK 74078. USA.

Siliques from 2900 transgenic families of Arabidopsis produced by Ken Feldmann following seed transformation with Agrobacterium tumefaciens were screened for aborted seeds. Over 75 families were shown in subsequent generations to be segregating for recessive embryonic mutations similar to those isolated following chemical mutagenesis. Two fusca mutants that accumulated anthocyanin in cotyledons were also found. Mutant families were subsequently analyzed for segregation ratios, patterns of developmental arrest, distribution of aborted seeds in heterozygous siliques, response in culture, nopaline production, ratio of kan^r:kan^s progeny, frequency of kan^r wild-type plants, and patterns of T-DNA insertion. Roughly 30% of these mutants are blocked at the globular stage of embryogenesis; another 30-40% reach a later stage of development, and the remainder are blocked early in development. Interesting phenotypes include the presence of highly abnormal suspensors, embryos with altered patterns of symmetry, mutant seeds with twin embryos, and bloated embryos with altered cell morphology. A putative female gametophytic mutant that produces 50% rather than 25% aborted seeds following self-pollination has also been identified. Previous studies with 18 mutants produced in a similar manner (Errampalli et al., Plant Cell 3: 149-157, 1991) suggest that approximately half of these mutants are tagged with T-DNA. We are currently using the approaches outlined above to identify tagged mutants with interesting patterns of abnormal development that merit further analysis at the molecular level. The long-term goal is to determine the functions of specific housekeeping and regulatory genes during plant embryogenesis.

POSTERS POSTERS POSTERS

A histological and elemental analysis study of the mature seed of Begonia semperflorens.

M. West and J.N.A. Lott, Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1.

The main focus of this study was to investigate the total element content and the subcellular distribution of mineral nutrients in a species that has very minute seeds. The major storage substances in the seed of Begonia semperflorens were determined to be lipids sequestered in lipid vesicles and proteins concentrated in protein bodies. Transmission electron microscopy revealed the presence of protein crystalloids and electron-dense globoid crystals in the protein bodies. These inclusions varied both in size and number in protein bodies from different tissues. Energy dispersive x-ray analysis of the globoid crystals showed the high Mg, K and P levels characteristically found in phytin from large sized seeds. Small traces of Ca were found in globoid crystals in the provascular tissue of the embryo. Neutron activation analysis was used to measure the elemental composition of the whole seed and these results were correlated with the findings of energy dispersive x-ray analysis studies of globoid crystals.

Variable Glycosylation of a Peanut Cationic Peroxidase.

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A cationic peroxidase, used extensively for studies of structure and function, has been characterized by formic acid cleavage and amino acid sequence determination as the protein expressed by the cDNA clone prxPNC1 (PNAS 87:8874,'90).

It had been noted that this isozyme may be occurring in two forms, CP^- and CP^+ being glycosylated with four glycans and with three glycans (Isozymes 315).This study gives additional strength to this argument by the findings that both forms not only have the same peptide mass but also have the same calcium content and exhibit the same heme absorption wavelength and the same specific enzyme activity. These parameters differ greatly between true isozymes such as the cationic and anionic isozymes of peanut peroxidase (Plant Cell, Tissue and Organ Culture 22: 65'90)

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Phenolic compounds as bioindicators of air pollution

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European pine (*Pinus nigra*) and American pine (*P. resinosa*) were tested for needle damage caused by atmospheric acid depositions and salt spray. Morphology and histology of the needles from outdoor plants were compared with those of 3- to 5-year-old plants growing in the greenhouse under controlled conditions. Potted plants covered with plastic bags were sprayed each day with either distilled water or one of the solutions: aluminum chloride, H_2SO_4 of pH 2.5-4.5, saturated NaCl solution. Needle tips, mid-parts and bases were fixed after 1h, 3h, 24h, 7 days and 30 days in glutaraldehyde + caffeine for precipitation of phenolics. Histochemical reactions and observations in the electron microscope showed that morphological damage is connected with increased deposition of phenolic compounds in the mesophyll cells, starting directly under the stomata. Disorganization of the membranes leads to the death of mesophyll cells, even before morphological damage is recognizable on the surface of the needle. Thus histochemical reactions allowed damage to be detected earlier than was possible by macroscopic observation. The results suggest that the reaction is nonspecific for a particular compound, but is the response of the cells to stress.

Pulse sugar release from tropical epiphyte communities (Bryophyta).

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The accumulation and release of sugars was examined in two bryophyte communities from upper montane rain forest in Guadeloupe. Sugar composition was determined by high-performance liquid chromatography and nuclear magnetic resonance spectroscopy. Total sugar and polyol reserves were equivalent to 17% of dry weight in the upper canopy hepatic Frullania atrata and 6% in the lower canopy moss Phyllogunium fulgens. Exposure to wetting/drying cycles resulted in the substantial loss of sugars to throughfall solution. Among sugars and polyols detected in collected leachate were fructose, mannitol, glucose, erythritol, glycerol and sucrose. The release of sugars and polyols during individual rewetting episodes was greatest in the lower canopy bryophyte community. However, the greater frequency of drought exposure in the upper canopy results in higher total release for the Frullania dominated community, estimated at 223 kg ha^{-1}. This compares to only 118 kg ha^{-1} from the lower canopy Phyllogunium mats. These release episodes potentially have significant impact on many aspects of nutrient cycling, particularly for processes of microbial decomposition and asymbiotic nitrogen fixation, each of which are readily influenced by the supply of exogenous sugars.

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Choline biosynthesis in spinach: Role and regulation of phosphorylethanolamine N-methyltransferase activity.

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Choline plays a ubiquitous role in plant lipid metabolism. In Chenopodaceae, Gramineae and representatives of several other families, choline also serves as a precursor for the synthesis of the organic solute betaine, a compatible osmolyte strongly implicated in the adaptation of plants to drought and salinity. In spinach, choline is synthesized via a pathway involving the sequential N-methylation sequence: phophorylethanolamine → phosphorylmonomethylethanolamine → phosphoryldimethylethanolamine → phosphorylcholine. The product of this sequence is modified by phosphatase action to produce choline. The N-methyltransferase (PEAMeT) which catalyses the first methylation step is believed to catalyse the rate-limiting step of this pathway. Our studies of PEAMeT show that this enzyme is present in the soluble fraction of both leaves and roots of spinach. PEAMeT activity in both tissues can be induced several-fold in gradually salinized or salt-shocked spinach. Also, the activity of PEAMeT *in vitro* can be completely inhibited by the addition of one of its reaction products, S-adenosylhomocysteine. We have also found that the activity of PEAMeT in spinach is light-regulated. The nature and level(s) at which PEAMeT activity is regulated by osmotic stress, light and reaction intermediates and products is currently being studied.